JOURNAL OF THE ROYAL SOCIETY Interface

J. R. Soc. Interface (2008) **5**, 1263–1278
doi:10.1098/rsif.2008.0142
Published online 22 July 2008

REVIEW

The combined role of wear particles, macrophages and lymphocytes in the loosening of total joint prostheses

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This review considers the causes of loosening of prosthetic joint replacement paying attention to the biological mechanisms rather than other effects that are physical, such as component fracture and other failure related to mechanical problems. Infection accounts for approximately 1.5 per cent of joint loosening and when it occurs it is a cause of serious concern to the surgeon. The loosening of prosthetic joints in the absence of infection is by far the most common reason for revision surgery and is known as aseptic loosening. While this may be multifactorial in terms of causation, and non-biological factors may contribute significantly in a particular individual, a significant part is undoubtedly played by the generation of wear debris, mainly from the bearing surfaces of the joint, and the cellular reaction to this in the implant bed. Phagocytic cells (macrophages and multinucleated giant cells) are the ones that remove foreign material from the tissues, and the ways in which these cells function in the interface between implant and bone are described. Mediators produced locally include numerous cytokines, enzymes and integrins. There is evidence for interactions between macrophages and locally recruited lymphocytes, which may or may not give rise to an immunologically mediated process.

Sensitization of individuals having metal implants in place has been shown by positive skin tests or blood lymphocyte transformation tests and in these cases has been accompanied by loosening and failure of the replacement joint. The question remains as to whether this process is also present in a proportion of individuals with aseptic loosening in the absence of clearly defined clinical evidence of sensitization.

Numerous studies performed by the author's group and, latterly, by others suggest that the cellular reactions detected in the tissues in cases of aseptic loosening are indeed those of contact sensitization. There is good evidence to show that a type IV cell-mediated immune reaction is taking place, with T_H1 cell involvement and active antigen presentation. The extent to which sensitization is present in individual cases of aseptic loosening remains a subject for further work and this needs all the sophisticated molecular methods now available to modern biology to be applied in appropriate prospective clinical studies coupled with experimental models *in vitro* and *in vivo*. Immunological processes may play a more important part in joint loosening than previously considered.

Keywords: joint replacement; implants; prosthetics; inflammation; macrophage; lymphocyte

1. INTRODUCTION

The total replacement of joints by the implantation of permanently indwelling prosthetic components has been one of the major successes of modern surgery in terms of relieving pain and correcting deformity. A broad view from the literature shows that 90–95 per cent of hip replacements are successful for 10–15 years, while knees are only a little less successful than this in terms of 10-year results. The failure of joint replacement occurs for a variety of reasons, which have been recently detailed elsewhere (Burke & Goodman 2008; Revell 2008*a*). Adverse effects may be divided into mechanical and biological categories. The former includes excessive wear at the bearing surfaces, recurrent dislocation, malalignment, stress shielding,

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This review article presents the main subject matter of the George Winter Award lecture delivered to the European Society for Biomaterials at the 21st European Conference on Biomaterials, Brighton, England on 12 September 2007 and titled 'Loose Joints'.

Received 11 April 2008
Accepted 30 June 2008

periprosthetic bone fracture and implant failure with or without fracture, while the latter basically comprises inflammation, albeit different types, which may occur as the response to infection or wear particles. In practice, there is a combination of physical and biological effects present by the time a joint requires revision surgery. Local osteoporosis, a form of disuse atrophy, may occur owing to altered loading of bone. Micro motion (movement at the micrometre level) is a further mechanical phenomenon that gives rise to a local tissue response (Revell 2008*b*). There are interrelationships between mechanical and biological effects in prosthetic joint loosening. The biological causes include infection but implant loosening in the absence of infection (aseptic loosening) is by far the largest problem. It is virtually impossible to separate mechanical and altered load-bearing effects from those related to the presence of large amounts of wear debris (see below) once the process of aseptic loosening is well advanced. Indeed, Mullhall *et al.* (2006) recently analysed the reasons for the failure of 318 knee replacements and found that 64.4 per cent had more than one identifiable cause. The following description will concentrate on the biological aspects of prosthetic joint loosening and will be confined to the inflammatory and immunological response to the presence of particulate wear debris in aseptic loosening. Infection and the possible alteration in the genome brought about by the continued presence of foreign material are important issues not considered in this review. The question arises as to whether the presence of foreign material in bulk or particulate form may give rise to tumours or developmental abnormalities.

The loosening of a prosthetic joint component in the absence of demonstrable infection is the most common reason for joint revision surgery. It is an open question as to whether the cellular reactions that occur near the implant are solely responsible for this form of joint failure. While abnormal wear at the bearing surface might give rise to excessive particle generation, the bone loss in relation to inflammation caused by these particles might in turn result in the loosening of the device and further abnormal mechanical loading. However, wear particles are now considered to be the major contributors to the development and perpetuation of aseptic loosening. A description of wear particle generation is not provided in this review, there being several other competent sources for the detailed tribology involved. But, it is necessary to mention the methods developed for the isolation of particles from tissues at the time of revision surgery and the ways in which this debris may be characterized as this gives important background information for the proper study and understanding of the cellular processes involved in bone loss and implant loosening.

2. WEAR PARTICLES: ISOLATION FROM TISSUES AND CHARACTERIZATION

There is inevitably some wear between any surfaces moving in relation one to another, whether in a machine or an artificial joint. An analogy with a car engine can be drawn in that there is a wearing-in period for prosthetic joints. Indeed, 'running-in wear' is a term used in joint tribology (Dowson 2008). That wear decreases with increased congruency and surface smoothness after this initial period has been shown in the implant retrieval studies of Yamac (1999). The size and morphology of polyethylene (PE) particles retrieved from the tissues in this series of hips was different in the first months after implantation compared with the longer term.

The appearances of various particles in the tissues viewed by conventional light microscopy have been described elsewhere (Revell 1982) and the literature has been comprehensively reviewed by Savio *et al.* (1994).

Briefly, PE is seen as small particles or larger shards, both of which are unstained (transparent) objects that are birefringent on polarization microscopy. The small particles are intracellular in macrophages, while larger particles and flakes are included within by foreign body multinucleated giant cells (MNGCs). Metal particles are seen as brown or black granules or even short needles, which are surrounded by a weakly birefringent halo on polarization microscopy, a feature attributed to the formation of metal proteinates at the surface of the particle (Revell 1982; Revell *et al.* 1997). Large shards of metal are not necessary for giant cell formation, since much smaller metal particles are also found in MNGCs as well as macrophages. Bone cement (polymethylmethacrylate, PMMA) is soluble in the solvents used in tissue processing for histology, and the sites where PMMA was present are therefore empty spaces containing foci of very fine granular material, the undissolved radiographic contrast material present in the cement.

Ceramic debris is usually less than 5 μm in diameter presenting as fine greyish-brown particles (zirconia) and brown, brownish-green or black granules (alumina). Mochida *et al.* (2001) provided further information about ceramic particles, their isolation and characterization. Separate fragments and particles of hydroxyapatite have been identified in tissues (Bauer *et al.* 1991) as have flakes and particles of polyether-etherketone polymer and carbon fibre-reinforced plastics (Revell 2006*c*).

It became apparent in the mid-1990s that the vast majority of wear particles generated by artificial joints and found in the tissues were submicroscopic and therefore not visible by light microscopy (Margevicius *et al.* 1994; Shanbhag *et al.* 1994; Campbell *et al.* 1995; Maloney *et al.* 1995; Doorn *et al.* 1998). Methods for the isolation of fine particles from tissue were developed with ultrastructural methods used for their characterization. This work focused initially on PE debris, but methods for isolating metal, ceramic and acrylic particles were soon developed (Doorn *et al.* 1998; Iwaki *et al.* 1999, 2000; Yamac 1999). Fine metal particles were isolated by Campbell and her group (Doorn *et al.* 1998) and Yamac (1999) among others. The recognition that metal particles may be as small as 10–70 nm (Case *et al.* 1994) and 6 nm (Doorn *et al.* 1998) and therefore defined as nanoparticles (Revell 2006*a*) is of importance as will be seen later. That there were also metal nanoparticles present in the samples examined by Yamac (1999) is also now apparent on review of the results in this work. Metal nanoparticles within implant interface macrophages are shown in

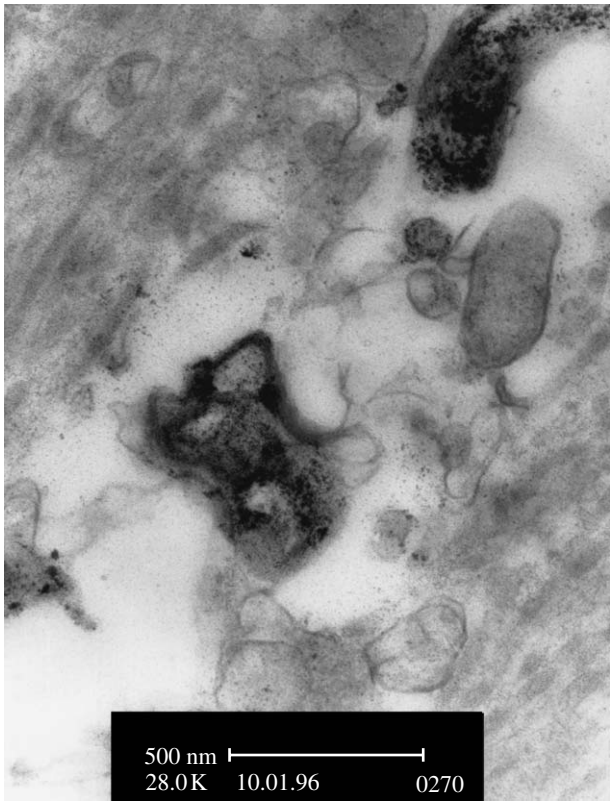

Figure 1. Transmission electron micrograph to show the presence of numerous nanoparticles of CoCr in aggregates within membrane-bound vesicles and pseudopodia of a macrophage in interface tissue retrieved at the time of revision surgery for the aseptic loosening of a hip joint (original magnification: 28 000×).

figure 1, which is a previously unpublished finding from work in our laboratory by Yamac.

It is clear that electron microscopy should always be used to characterize the wear particles, but in practice, even those who have developed the isolation methods, including our own group, have not used them in parallel with detailed analyses of inflammatory and immunological mechanisms. The assumption is made that there are numerous micro- and nanoparticles in the infiltrating cells at the implant interface but it is simply not known which particles are present in tissues near to a loosened joint on which sophisticated immunohistochemical, molecular biological and proteomic methods have been applied. However, the effects that various implant materials in nano- and microparticle forms have on macrophages in cell culture has been examined using techniques of varying degrees of sophistication. These functional studies bring credibility to the histological and tissue extraction methods used on interface tissues, which show the changes *in situ* described later in this article. The large literature on cell culture studies including the use of cell lines will not be included here since this could itself form the basis for a separate major review.

3. THE FATE OF PARTICLES DERIVED FROM PROSTHETIC COMPONENTS

The bearing surfaces of a replacement joint are the main site of wear and the particles generated are shed directly into the synovial fluid from which they are removed by macrophages in the fluid and the synovial lining cell layer. Other parts of the prosthesis may give rise to particles, for example, by abrasion of the stem of a femoral component or the wear of a trunnion between the femoral head and the upper part of a femoral stem. In addition, there may be wear and/or corrosion around screws. Specific details of wear mechanisms will vary with particular joints including the spine and cannot be included here. The main thrust of this account is to describe the effects of those particles presenting initially in the joint fluid, with the hip and knee as the exemplars, and the consequences that result from their distribution deep within the peri-implant bone as well as at distant sites in the body. To fully understand this, it is necessary first to consider the morphology of the joint lining, i.e. the synovial membrane, in the normal and replaced joint.

4. SYNOVIAL LINING CELLS OF JOINTS AND AT THE IMPLANT–BONE INTERFACE

The normal diarthrodial joints such as the hip and knee are contained within a fibrous capsule, the inside of which is covered by a layer of specialized cells that produce the synovial fluid. This fluid is responsible for cartilage nutrition and the removal of metabolites from the joint as well as joint lubrication and the provision of a milieu for the cells (mainly polymorphonuclear leucocytes, macrophages and lymphocytes) that are involved in defensive processes, including the removal of foreign material and organisms from the joint. The joint-lining layer of cells, often called synoviocytes or simply synovial lining cells, increases in thickness through an increase in the number of cells present. This non-specific response occurs in various joint conditions, including inflammatory arthritis, marked degenerative joint disease and crystal-induced disease such as gout. This process is frequently called hyperplasia (an increase in the number of cells by proliferation occurring as a response to increased functional demand), but strictly this is not a hyperplasia, because proliferation of the synovial lining cells in general does not occur. The exception to this is that occasional basally situated type B cells divide, as shown by Lalor *et al.* (1987).

The cells lining the joint were classified into type A and type B synoviocytes with the B cells situated deep to the A cells on the basis of ultrastructural studies (Barland *et al.* 1962; Ghadially 1983; Graabeck 1988). The type A cells were labelled with macrophage monoclonal antibody (MAB) markers at light and electron microscopical levels (Forre *et al.* 1982; Hogg *et al.* 1985; Palmer *et al.* 1985; Mapp & Revell 1988). Fibronectin was localized to the deep part of the synovial lining cell layer and was shown to be produced by type B synoviocytes in light microscopy and transmission electron microscopy immunocytochemical studies (Mayston *et al.* 1984; Mapp & Revell 1985). Type IV collagen, laminin, chondroitin sulphate, heparan sulphate, type V collagen and entactin have all been localized to the deep B synoviocyte region of the synovial lining (Pollock *et al.* 1990; Revell *et al.* 1995) and all of these molecules are components of, or closely associated with, the basement membranes of

epithelia. Although there is no true basement for the synovial lining, one function of type B synoviocytes seems to be the provision of local anchorage for the overlying phagocytic type A synoviocytes. Evidence for the production of these molecules by type B synoviocytes comes from their localization to the endoplasmic reticulum of these cells in ultrastructural studies (Revell *et al.* 1995). A further monoclonal antibody (MAB 67) was also found to be a marker of type B synoviocytes by Stevens *et al.* (1990). Other molecules localized to the synovial lining include cellular adhesion molecules (CAMs; intracellular adhesion molecule, ICAM-1; vascular cell adhesion molecule-1, VCAM-1) and CD44 (Hale *et al.* 1989; Koch *et al.* 1991; Wilkinson *et al.* 1993; Henderson *et al.* 1994; Szekanecz *et al.* 1994).

The synovial lining is removed at the time of joint replacement surgery but a lining of cells indistinguishable from the normal joint lining grows back again. This is frequently referred to as the pseudo-synovium in the orthopaedic literature, though the author finds no reason why it should not simply be called the synovial membrane. There is much evidence that a synovial lining forms in the body under various circumstances, e.g. the lining of acquired bursae in soft tissue and that of the air pouch induced on the back of rats in an experimental model used for inflammation research (Edwards *et al.* 1981). Goldring *et al.* (1986) showed that a synovium-like structure is present at the surface of the fibrous tissue between implant and bone using routine light microscopy. That this cellular layer is similar to, if not identical with, true synovium has been shown by detailed immunohistochemistry studies in which macrophages and fibroblasts are present and distributed just like the type A and type B cells of the true synovium (Revell & Lalor 1991). Identical with that of true synovium is the localization of fibronectin, type IV collagen and laminin around the deeper fibroblastic cells, which are in addition marked with MAB 67 (Lalor & Revell 1993*a*). Type V collagen and heparan sulphate are also present (P. A. Revell 1992, unpublished findings), as are ICAM-1, VCAM-1 and CD44 (Al-Saffar *et al.* 1995*a*; McFarlane & Revell 2004). Macrophages and MNGCs have been noted to be present in small numbers at the interface between tissue and the bulk material of a well-fixed implant, for example, in relation to bone cement (Levack *et al.* 1987), but it is the recruitment of large numbers of these cells in response to the presence of wear debris, it is posited, which gives rise to the synovium-like appearance seen where there is aseptic loosening. Section 5 will consider the spread of particles from the bearing surfaces of the artificial joint to other sites.

5. WEAR PARTICLE DISSEMINATION FROM THE JOINT TO DISTANT SITES INCLUDING THE BONE AROUND THE IMPLANT

The bearing surfaces of the artificially replaced joint are bathed in synovial fluid into which wear particles from the articulation are shed. There are phagocytic cells in suspension within the synovial fluid and, as has been seen, lining the joint as the synovial membrane. If small amounts of debris are shed into the joint fluid, these can be removed by the phagocytes and cleared through the lymphatic vessels draining the joint to the local lymph nodes (Vernon-Roberts & Freeman 1976; Revell 1982, 1986). There has long been an assumption that there are lymphatic vessels around joints, but these have only recently been identified for certain in true synovium and in the implant–bone interface using immunohistochemical labelling (Jell *et al.* 2006). Lymph nodes filter foreign material out of lymphatic fluid and the spleen does the same for the blood. The identification of the products of wear and corrosion in lymph nodes has been noted by numerous workers including Langkamer *et al.* (1992), Case *et al.* (1994) and Bae *et al.* (1996). A total of 15 separate references to biomaterial-related lymph node enlargement is available in the review by Al-Saffar & Revell (1999). Dissemination of biomaterials from joints to other organs has also been reported, namely the spleen, liver, kidney and lung (Bauer *et al.* 1993; Case *et al.* 1994; Urban *et al.* 2000). Experimentally, CoCr particles have been disseminated from the lower femur to the spleen in an animal model (Revell *et al.* 2004). That traces of implanted materials are present in distant organs is beyond doubt. The effects that there may be from such dissemination of material will be treated in §10 of this review after consideration has been given to likely immunological processes.

The means by which particles reach the bone–implant interface need to be addressed, since at this location they cause a cellular response that damages the bone and so alters the fixation of the prosthesis. The question arises as to whether there is continuity between the joint and the microscopically small zone between the implant and the bone surrounding it. With the exception of bioactive (hydroxyapatite) coatings of implants, where there is bone ongrowth and integration, the tissue, be it bone or fibrous tissue, is discontinuous with the implant and there is a true interface in material terms. The true synovial surface is bathed in fluid and it seems likely that the synovium-like layer next to an implant also has fluid between it and the man-made material. Also, it seems likely that there is continuity between this fluid and that of the nearby joint. Such continuity around the cemented prosthetic hip has been shown in human bone specimens (Liebs *et al.* 1997), while the effects of fluid pressure in the immediate peri-implant region on the migration of particles deep within the bone have been discussed by Aspenberg & Van der Vis (1998). Incidentally, Anthony *et al.* (1990) suggested that particles could track between the metal implant and bone cement, finding their way out through fine cracks in the cement mantle to the peri-implant tissue.

Gruen *et al.* (1979) divided the area around a replaced hip joint into seven zones on anteroposterior radiographs and evaluated the sites where changes were present. Radiolucency between implant (cement) and bone was seen most in zone 1, then in zone 4, which is situated near the lower end of the stem. The frequency of changes was more or less equal in all the other zones, with a slight predominance for the medial rather than the lateral ones. If there is excessive wear and an accumulation of particles beyond the amounts that can

be removed by local macrophages through the lymphatics, then the local erosion of bone due to the cellular reaction to debris would be expected to be in the proximal parts of the bone, nearest to the joint, i.e. in zones 1 and 7. The occurrence of bone loss deeper in the bone (zone 4) suggests that debris is conducted there in the fluid bathing the implant. Deep areas of osteolysis might be due to the fragmentation of bone cement at this level, and indeed, cement particles may be released into the bone–cement interface causing a phagocytic response and local bone loss (see below). However, radiolucency is seen in relation to cemented implants in the absence of cement particles.

Numerous descriptions refer to the presence of PE particles deep within the bone adjacent to cemented or uncemented metal prosthetic joints. Such particles must have originated from the acetabular component of the hip. Distribution to deep sites around the femoral stem is likely to be in the fluid bathing the implant as described above. The encroachment of bone resorption from the periphery of the acetabular component in relation to macrophage activity in response to PE debris was noted by Schmalzried *et al.* (1993), though they considered that femoral radiolucencies were due to stress-related bone remodelling. It now seems more likely that bone loss around the femoral component at the hip occurs in relation to PE particles, though, of course, a contribution by mechanical processes cannot be completely excluded. Kobayashi *et al.* (1997) isolated and quantified the PE particles in interface tissue samples from areas of osteolysis comparing the amounts with those where there was no osteolysis. There was a statistically significant difference and osteolysis was present when PE particles were in excess of 1×10^{10} per gram of tissue. A detailed bibliography on the effects of PE wear debris as well as the tribology of excessive PE debris generation is provided elsewhere by Jin & Fisher (2008).

Metal particles were not considered problematic when most total joint replacements had metal against PE (M-PE) articulations, but they have come into prominence since the introduction of second-generation metal–metal joints, such as the resurfacing hip arthroplasty. The particles generated from metal–metal hip articulations are nanoparticles, both from isolation studies (Doorn *et al.* 1998) and joint simulators (Brown *et al.* 2007). The smaller a particle the greater is its surface area relative to its volume and thus the greater its chemical reactivity. It is this nanoparticulate element to the wear debris which has caused concern. That metal particles were present in considerable amounts where metal articulated against PE is apparent in the literature, which includes the use of the term 'metallosis' to describe the black discolouration of tissue due to overloading with metal. Many metal–PE prosthetic joints have metal also used in sites other than at the bearing surface, like a metal back to the acetabular cup or a metal tibial tray underlying a PE tibial plateau. Fretting corrosion of screws and metal backs was identified by Yamac (1999) in a large number of ostensibly metal–PE hip prostheses removed for aseptic loosening.

6. PHAGOCYTOSIS: THE BIOLOGICAL RESPONSE TO PARTICULATE WEAR DEBRIS

The cells that deal with foreign material are the phagocytes comprising the macrophages and MNGCs, the latter being formed by the fusion of macrophages. Macrophages are present wherever there are wear particles, and when abundant give rise to the histological appearance of the synovial membrane and the thickened synovium-like layer in the bone surrounding the prosthetic component. Large accumulations of phagocytic cells are responsible for the removal of bone described as osteolytic areas in the radiograph. Wear debris may be apparent within these cells but the greater proportion of particles is not visible by light microscopy (see above). Macrophages and MNGCs may be present in a diffuse distribution or organized into localized nodule-like collections termed granulomata. A granuloma has accompanying lymphocytes usually as a surrounding cuff and is characteristic of certain diseases in which cell-mediated immunity is involved, such as tuberculosis, and thus its recognition has clear diagnostic implications to the cellular pathologist. The term granuloma is unfortunately used more loosely in the biomaterials and orthopaedic literature often merely to mean a macrophage and MNGC infiltrate. While true granulomas are seen in relation to debris and orthopaedic implants, this is not always the case. The term granuloma will not be used in the following description of the cellular pathology of bone loss in relation to wear debris, not because granulomas do not occur, but because the loose use of the term does not permit clear comparisons of the descriptions in the literature.

Routine haematoxylin–eosin staining of tissue sections is not sufficient for the recognition of cells because the morphology of any cell can be misleading when considered in isolation. Monoclonal antibodies (MABs) recognizing molecules on the surface (epitopes) of cells or particular substances within them may be used to characterize cells using immunocytochemistry. Such markers enable the recognition of not only cell type but also the functional activity. The MABs are categorized according to the epitope that they recognize according to CD numbers. CD refers to 'cluster of differentiation'. For example, CD68 is a 110 kDa transmembrane glycoprotein present in circulating monocytes and tissue macrophages, which is also present in MNGCs. CD13, CD35 and CD36 are also macrophage/MNGC markers. There are numerous studies that show the abundant presence of macrophages and MNGCs in the periimplant tissues using these markers where there is wear debris present (Revell *et al.* 1997; Al-Saffar & Revell 1999; Revell 2006*b*). That some of these cells are activated in the implant interface has been shown by the recognition of surface HLA class II molecule (HLA-DR; Al-Saffar & Revell 1994; Revell & Jellie 1998) and integrin expression (Kadoya *et al.* 1997; Clarke & Revell 2001), especially CD11b (αM/β2 integrin). Cell culture studies by Curtis (2002) and Altaf (2007) have shown these molecules to be expressed by macrophages when they engulf wear particles. Clarke (1999) demonstrated the expression of various cytokines

Table 1. Cytokines demonstrated in tissue adjacent to orthopaedic implants with references.

interleukin-1α	Westacott *et al.* (1992)
interleukin-1β	Dorr *et al.* (1990), Westacott *et al.* (1992), Jiranek *et al.* (1993), Al-Saffar & Revell (1994), Chiba *et al.* (1994), Al-Saffar *et al.* (1995*b*) and Goodman *et al.* (1998)
interleukin-6	Chiba *et al.* (1994), Al-Saffar *et al.* (1995*b*) and Goodman *et al.* (1998)
interleukin-10	Merkel *et al.* (1999), Hercus *et al.* (2002), Altaf & Revell (2004), Altaf *et al.* (2006) and Altaf (2007)
interleukin-11	Xu *et al.* (1998)
interleukin-15	Revell & Jellie (1998) and Saeed & Revell (2001)
tumour necrosis factor-α	Chiba *et al.* (1994), Al-Saffar *et al.* (1995*b*), Goodman *et al.* (1998) and Merkel *et al.* (1999)
transforming growth factor-α	Al-Saffar & Revell (2000) and Xu *et al.* (2000)
granulocyte-macrophage colony-stimulating factor	Al-Saffar *et al.* (1996)
macrophage colony-stimulating factor	Xu *et al.* (1997) and Al-Saffar & Revell (2000)
platelet-derived growth factor	Jiranek *et al.* (1993)
epidermal growth factor	Bainbridge & Al-Saffar (1998) and Xu *et al.* (2000)

by macrophages during particle phagocytosis *in vitro*. The literature describing cytokine production by interface macrophages is large but a good source of references is the article by Archibeck *et al.* (2000). Those cytokines that have been found in periprosthetic tissues in macrophages containing wear particles are summarized in table 1, which also provides details of the relevant references. Prostaglandin E2 (Goodman *et al.* 1989; Dorr *et al.* 1990), various metalloproteinases/collagenases (Goodman *et al.* 1989; Dorr *et al.* 1990; Vidovszky *et al.* 1998; Al-Saffar & Revell 2000) and inducible nitric oxide synthase (Moilanen *et al.* 1997) have also been shown in association with the macrophage infiltrate in relation to wear debris.

These mediators are for the most part pro-inflammatory, one exception being IL-10, and their involvement in the inflammatory process is extremely complex, with various interactions taking place. A detailed analysis could itself form the basis of a further review article and any attempt at further description will not be attempted here. A brief account of the way that some of these cytokines contribute to osteoclast production and the relationship between osteoclasts and cells of the macrophage/MNGC type will be provided in §7, mainly because it is these cells that are responsible for the resorption of bone, and therefore may contribute significantly to the development of implant loosening.

7. OSTEOCLASTS, MACROPHAGES AND MNGCs: THE CELLS INVOLVED IN BONE LOSS IN RELATION TO WEAR PARTICLES

Some cytokines, TGF-α, M-CSF and GM-CSF, have direct effects on both osteoclast and MNGC formation, with M-CSF and TGF-α mainly influencing osteoclastogenesis while GM-CSF promotes MNGC formation. Other cytokines, IL-1, TNF-α and IL-6, indirectly influence osteoclasts through their action on osteoblasts as well as the macrophage-driven cellular reaction that results in bone loss.

The osteoclast is the specialized cell responsible for the removal of bone in normal metabolism and various pathological processes. It is, therefore, likely that a significant amount of the bone loss occurring in the osteolysis associated with the aseptic loosening of an implant is mediated by the classical bone resorptive activity of osteoclasts. However, there is some evidence that macrophages and MNGCs may also be capable of bone removal (figure 2). Most of the work looking at cellular infiltrates at the bone–implant interface has not actually included the bone, but merely the tissue stripped from the bone. Important are the studies by Kadoya who did obtain small amounts of bone as well as the interface soft tissue at revision surgery. He demonstrated that not only osteoclasts occupied 7.67 ± 1.82 per cent of the bone surface, but also macrophages covered 19.33 ± 5.16 per cent of the surface (Kadoya *et al.* 1996). Comparing tissue retrieved from sites with radiographic evidence of osteolysis and those without osteolysis showed significantly higher macrophage surface in the former (33.37 ± 8.59 versus $5.29 \pm 1.34\%$; Kadoya *et al.* 1997). There were also significantly more osteoblasts on the bone surface in osteolytic zones compared with non-osteolytic, a finding that may reflect the effects of pro-inflammatory cytokines mentioned above, or even represent an attempt at bone formation and repair in the presence of resorption. That osteoblastic and osteoclastic activities are coupled in bone metabolism is well recognized, but there is greater

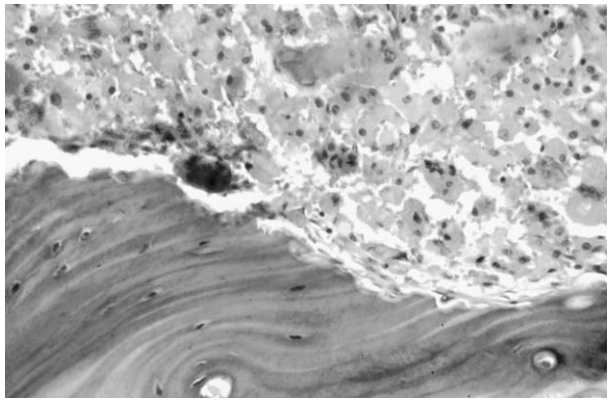

Figure 2. High power light microscopy photomicrograph of a macrophage and MNGC infiltrate, which is in continuity with surface of bone. Note the crenated appearance of the edge of the bone due to resorptive activity and the way in which the lamellar structure is cut across at the surface. A single osteoclast is present in the left centre.

complexity to these interactions in pathological processes that occur in the presence of foreign particles. The likely additional involvement of lymphocytes in these processes is briefly mentioned at the end of this section.

While it has long been held that macrophages/MNGCs and osteoclasts have a common lineage being derived from a common bone marrow precursor cell, it has also been debated how far back in this lineage the separation of cell types might be in terms of their differentiation and functional capabilities. Kadoya showed that some of the markers for osteoclasts were also shared by MNGCs in the infiltrate on the bone-related part of the interface, with those MNGCs on the implant side of the interface tissue and in relation to the synovium-like layer not expressing these markers (Kadoya *et al.* 1994). MNGCs on the bone surface had a ruffled border in relation to the bone by transmission electron microscopy, and were thus like osteoclasts, but the same cells also contained submicrometre-sized metal particles in cytoplasmic membrane-bound bodies (Kadoya *et al.* 1997). It seems likely, then, either that osteoclasts are capable of particle engulfment, or that MNGCs containing wear debris are able to resorb bone. Further discussions on the definition and differentiation of cell type are semantic and unhelpful in this context. Athanasou and his co-workers have shown that debris-related macrophages are capable of resorbing bone *in vitro*, and that osteoclasts may themselves be capable of biomaterial particle phagocytosis (Athanasou *et al.* 1992; Quinn *et al.* 1992; Wang *et al.* 1997).

The signalling system between osteoblasts and osteoclasts involves the hitherto unmentioned molecules known as receptor activator of nuclear factor-κB (RANK) and its ligand (RANKL). These molecules play a central role in osteoclastogenesis and osteoclast activation and have been recently reviewed by Boyce & Xing (2007). RANKL is present in the interface tissues (Haynes *et al.* 2001; Mandelin *et al.* 2003). Furthermore, interleukin-17 (IL-17) is produced by T helper lymphocytes in the implant interface where there is aseptic loosening (Hercus *et al.* 2002; Hercus 2005), the significance of this observation being that this cytokine stimulates RANKL expression and hence promotes osteoclastic activity. The presence of T lymphocytes in relation to loosened prostheses and possible involvement of the immune system will be considered in the remainder of this review.

8. LYMPHOCYTES IN THE BONE–CEMENT INTERFACE, SENSITIZATION AND ASEPTIC LOOSENING

Lymphocytes were noted to be present in the tissues associated with loosened joint implants over a quarter of a century ago (Vernon-Roberts & Freeman 1976), but regarded as either a rare occurrence or their presence to be of little consequence since they were thought to be present in such small numbers (Jasty *et al.* 1986; Jiranek *et al.* 1993). Working in the author's group in London, Lalor recognized the likely significance of a lymphocytic infiltrate at the implant interface suggesting an association with an identifiable immunological process, namely type IV sensitivity (cell-mediated immunity and contact sensitization; Lalor *et al.* 1990, 1991).

The lymphocytes in the infiltrate seen in relation to aseptically loosened hip joints were identified as T cells using immunohistochemistry and there were no B cells or plasma cells present (Lalor *et al.* 1990, 1991; Lalor & Revell 1993*a*,*b*). Two of the individuals were shown to be sensitized to titanium on skin testing, and the others were considered also to be sensitized even in the absence of such a positive clinical dermatological test. Titanium sensitization although very rare has been reported since in relation to joint replacement (Parker *et al.* 1993; Case *et al.* 1994).

The presence of T lymphocytes and the lack of B cells has been a consistent and recurring theme of our work ever since these pioneering studies. When an immune reaction occurs in the body, it may have local tissue damaging or systemic effects known as hypersensitivity reactions. Those reactions mediated by B cells have been called types I, II and III hypersensitivities, while type IV hypersensitivity is mediated by T lymphocytes. The finding of only T cells has caused us to propose, and continue to seek evidence for, an immunological reaction in the presence of wear debris.

The idea that sensitization to metals occurs in some patients with a joint replacement was not new in the 1990s since cases had been described 20 years earlier, mostly in first-generation metal against metal hip replacements. Skin sensitization tests on 50 patients with metal against metal hip joints by Elves *et al.* (1975) found that 38 per cent of them were sensitive to one or more metals tested (Cr, Co, Ni, Mo, V and Ti). Of 23 individuals with joint loosening, 15 were sensitive, leaving 4 sensitive cases out of the remaining 27 who did not show loosening and it was concluded that metal-on-metal implants may cause sensitization. In the same issue of the journal, Benson *et al.* (1975) reported an unexpectedly high incidence of metal sensitivity on skin testing of patients with metal-to-metal hip arthroplasties (28%) compared with those having metal-to-plastic prostheses (2.6%), the latter having no higher incidence of metal sensitivity than a control group awaiting operation. A small number of individuals had been reported a year earlier as showing elevated levels of Co and Cr in tissues adjacent to CoCr–CoCr articulations (Evans *et al.* 1974). This is one of the few papers at that or any other time in which histological appearances of periprosthetic tissues from sensitized individuals are described, and the authors noted tissue and bone necrosis in association with endarterial obliterative changes, as well as a macrophage and MNGC reaction. Of 14 individuals, 9 with loosened implants were sensitized having 11 loose implants between them, whereas no sensitivity was present in 24 individuals with well-fixed prosthetic joints.

That metal sensitization occurs in relation to metal on metal replacement joints seems to be the current received wisdom in the orthopaedic community. But, sensitization to metal where there is a M-PE articulation has been known for a considerable time. Thus, Nater *et al.* (1976) found metal sensitization in 4 out of 66 individuals with metal–plastic hip joints on skin testing, and even showed that one of these had been

negative before surgery. None of these individuals showed joint loosening. There are other accounts of metal sensitization in relation to M-PE joints (Lalor *et al.* 1990; Case *et al.* 1994) and it is a common finding that metal particles are present in the tissues related to M-PE implants even where PE is the predominant wear material (e.g. Pazzaglia *et al.* 1985; Kadoya *et al.* 1996, 1997). Those handling acrylic material, such as orthopaedic surgeons and dental technicians, are known to become sensitized, and cases in patients with cemented joint replacements have been described (Romaguera *et al.* 1985; Waterman & Schrik 1985; Gil-Albarova *et al.* 1992). Sensitization may be to *para*-toluidine, which is present in bone cement rather than to the acrylic itself. In all the cases of sensitization seen personally, be it to metal or acrylic, the predominant feature on histological examination of the tissues was a large increase in the number of lymphocytes. Subsequent study of tissue from the same sensitized individuals obtained after a period without an implant, when a two-stage revision operation has been performed and a 'spacer' been in place, shows that the T lymphocytes were no longer present. The question then is whether clear evidence of the type of immunological reaction taking place can be obtained using modern molecular and cell pathology investigative techniques.

9. EVIDENCE FOR IMMUNOLOGICAL PROCESSES IN LOOSENING: CHARACTERIZATION OF THE LYMPHOCYTES

The author and his group have long held the position that lymphocytes in the bone–implant interface in aseptic loosening are of major importance. These cells are seen as a diffuse infiltrate mixed with the macrophages and MNGCs and in aggregations having a perivascular distribution (Lalor *et al.* 1991; Lalor & Revell 1993*b*; Revell *et al.* 1997; Al-Saffar & Revell 1999; Revell 2006*b*), observations that have recently been confirmed by Willert (Davies *et al.* 2005; Willert *et al.* 2005). Labelling studies with anti-CD2 and/or anti-CD3 MABs show these lymphocytes to be T cells with no B lymphocytes present (reviewed by Revell 2006*b*), though Willert *et al.* (2005) show B cells present in periprosthetic tissues, albeit these are synovial membrane samples and not from the implant interface. In addition, this author describes the presence of plasma cells as do Milosev *et al.* (2006). The recognition of plasma cells in these studies relies on routine haematoxylin–eosin staining. In the absence of infection, they have not been a feature of the thousands of samples viewed by the author. It is suggested here that there might be an infective background in those cases where plasma cells are found or the cells may be mast cells that are sometimes difficult to tell apart from plasma cells in the absence of special stains. The recognition of mast cells in the interface has been made by immunohistochemistry with specific antibodies and they are frequently present in significant numbers (Al-Saffar *et al.* 1998).

The percentages of T cells in the interface tissues in aseptic loosening have been counted in two independent studies and calculated as 6–16 per cent (Hercus 2005) and 4–23 per cent (Altaf 2007). Two cases of known nickel sensitivity showed 30 and 31 per cent lymphocytes (Hercus 2005).

9.1. Subtypes of T lymphocyte

There are different types of T lymphocytes that are recognized by their functional activity. The two main groups are the T helper (T_H) and T cytotoxic/suppressor ($T_{C/S}$) cells, though the former have been divided into further subtypes known as T_H1 and T_H2 cells. There is a predominance of T_H cells over $T_{C/S}$ cells in the interface in aseptic loosening according to immunolabelling studies (Hercus *et al.* 2002; Hercus 2005) and the $T_H : T_{C/S}$ ratio has a mean of 7.2 : 1 (Hercus 2005).

The T helper cell subtype recognition could aid in the recognition of the sort of immunological process taking place in the interface, T_H1 cells being critical in macrophage activation and the T_H2 response being engaged in B cell activation and humoral immunity. $T_{C/S}$ cells are involved in the cell-mediated immune response. No evidence of a particular T helper cell subtype predominance was found by Arora *et al.* (2003) in tissue from areas of osteolysis. Overall, 10 per cent of the cells were T lymphocytes, which is a little lower than the findings quoted above (Hercus 2005; Altaf 2007), but in keeping with Jiranek *et al.* (1993). Employing the polymerase chain reaction (PCR) to characterize the cytokines present in interface tissue and provide a profile of the subtypes gives a different result. Such work demonstrates clearly that there is a predominance of T_H1 over T_H2 cells (Hercus & Revell 2001; Hercus *et al.* 2002), which is in agreement with results obtained by Weyand *et al.* (1998). The T cell subtype is determined by recognizing particular cytokines in the cells, including IFN-γ, TNF-β, IL-2 and IL-12 for T_H1, and IL-4, IL-5, IL-6 and IL-10 for T_H2 cells. IFN-γ and IL-10 provide useful markers of the two subtypes (Hercus *et al.* 2002). Protein extraction and western blotting of bone–implant interface samples have additionally shown the presence of IL-17, fractalkine and CD40, which are molecules having associations with T_H1 activity (Hercus *et al.* 2002). It may be seen from these results of T lymphocyte subtyping in implant interface tissues that a cell-mediated or contact sensitization process is likely to be taking place in the numerous cases of aseptic loosening examined. The latest contribution to the literature on T cell subsets in individuals having metal implants in place and comparing them with healthy controls showed the presence of T_H1 cells in the former (Hallab *et al.* 2008), confirming the findings of our group as outlined above.

9.2. The activation and proliferation of T lymphocytes

Histological examination typically and traditionally allows only the description of morphological changes present in tissues and cells, but modern techniques in which the expression of particular molecules is demonstrated, albeit as a snapshot in time, permit some insights into pathophysiology, especially when parallel

functional studies of the same cells and tissue are carried out *in vitro*. Thus, the expression of cytokines and cell adhesion molecules by macrophages on phagocytosis of particles may be studied in cell culture and the same molecular markers demonstrated *in situ*. This approach is clearly as valid for lymphocytes as it is for macrophages. The occurrence of an active immune process in relation to wear debris should then be demonstrable in the interface tissues and that process should be capable of being modelled in *in vitro* and *in vivo* experiments. This approach has been the cornerstone of the work characterizing immune processes in relation to particles in the author's laboratory.

The lymphocytes present in the interface have been shown to express HLA-DR and so are considered to be activated. A proportion of these cells are proliferating, in that they show the presence of the nuclear protein found in dividing cells, which is marked with MAB Ki67 (Revell & Jellie 1998). The proliferation of T lymphocytes and their maintenance is dependent on IL-2 *in vitro* and *in vivo*, so that a failure to find IL-2 in the implant interface could be taken as meaning those lymphocytes that are present cannot be involved in any immunological process. Indeed, a Finnish group suggested this, having found that T cell numbers were low and that there was no evidence of T cell activation as judged by a lack of IL-2, IFN-γ and TNF-β production by these cells and an absence of interleukin-2 receptor (IL-2R) expression (Li *et al.* 2001). As has already been mentioned above, there is evidence of IL-2, IFN-γ and TNF-β production when PCR methods are used. It happens that there is also a molecule, interleukin-15 (IL-15), which is an alternative to IL-2, and that this can perform the role undertaken by IL-2 in maintaining T cells both in culture and *in vivo*. This cytokine has been demonstrated to be present in large amounts in both macrophages and MNGCs in the interface membrane of aseptic loosening both as product (by immunohistochemistry) and message (mRNA; by PCR; Revell & Jellie 1998; Saeed & Revell 2001). Furthermore, the IL-2 receptor was demonstrable on some lymphocytes (Saeed & Revell 2001) and the mRNA for IL-2 could be found even though the cell product was difficult to show (Hercus & Revell 2001). Interface macrophages and MNGCs have an autocrine feedback mechanism using IL-15 and its receptor (IL-15Rα), whereas lymphocyte interactions with these phagocytic cells are mediated through the IL-2 receptor β (IL-2Rβ) on the cell surface (Saeed & Revell 2001). It is posited that direct cell-to-cell contact is necessary for both macrophage–macrophage and macrophage–lymphocyte interactions to take place in the context of cell activation by IL-15, the evidence for this coming from a combination of MAB labelling, SDS–PAGE, western blotting and RT-PCR methods (Saeed & Revell 2001). The importance of direct cell-to-cell contact and interaction will also be seen in §9.3 that treats another aspect of activation, namely antigen presentation.

9.3. T cell memory and antigen presentation

Lymphocytes pass from being naive cells to cells having so-called memory for an immune stimulus during the development of active immunity. Once again MAB markers enable recognition of the status of individual cells in this respect so that lymphocytes that are positively labelled with MAB against CD45RO (CD45RO positive; memory or primed cells) may be distinguished from naive cells that instead express CD45RA (CD45RA positive). The lymphocytes in the bone–implant interface in aseptic loosening are CD45RO-positive cells (figure 3) and are therefore primed cells (Revell & Al-Saffar 1994). The means by which lymphocytes become primed involves close cell-to-cell cooperation between specialized phagocytes and the lymphocytes in a process known as antigen presentation. The antigen-presenting cells (APCs) are found in lymphoid tissue (namely in lymph nodes and spleen) where they are called dendritic cells, as well as in the skin. Macrophages without a dendritic morphology may also show evidence of antigen presentation in some sites including the interface membrane as shown below. There are receptors and counter-receptors (ligands) known as the co-stimulatory molecules on the surfaces of the interacting cells and these have to be engaged in specific ways for antigen presentation to take place. Details of these molecules are given in table 2.

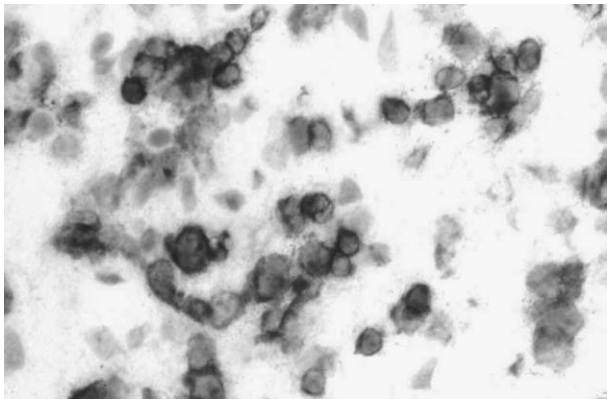

Figure 3. Lymphocytes in the interface tissue adjacent to an aseptically loose joint prosthesis, labelled with a MAB against CD45RO using an immunoalkaline phosphatase technique. The positive cells show surface labelling (black) and are memory (or primed) T cells.

On phagocytosing foreign material, not only does a macrophage produce various integrins and cytokines, but it also expresses large quantities of major histocompatibility complex (MHC) class II molecules on its surface, which is one of the features of macrophage and MNGC activation. MHC–peptide complexes on the surface of the cell are recognized by T lymphocytes, the complex engaging with the T cell receptor (TCR) and providing the first signal of antigen presentation. In the absence of any combination between these molecules expressed on the macrophage and the lymphocyte, there is no further propagation of any immunological process. Successful antigen presentation by the phagocytic cell with the activation and response by the T cell depends on the presence of a second signal, which is provided by the co-stimulatory molecules. These are pairs of surface molecules expressed on the surfaces of the APC and T cell, and antigen presentation occurs only when both primary and secondary signals are present. While various signalling molecules may be involved (table 2),

Table 2. Co-stimulatory molecules on the surface of macrophages/APCs and lymphocytes. (In brackets alternative names or full names of individual molecules are given.)

antigen-presenting cell	CD4-positive T lymphocyte
CD80/CD86 (B7-1/B7-2)	CD28
CD80/CD86 (B7-1/B7-2)	CTLA-4/CD3 (cytotoxic T lymphocyte antigen 4)
MHC class II–peptide complex	TCR (T cell receptor)
CD40	CD40L (CD154)
LFA-3 (lymphocyte function-associated antigen 3)	CD2
ICAM-1 (intercellular adhesion molecule 1)	LFA-1 (lymphocyte function-associated antigen 1)

the CD80/CD86 molecules on the phagocytic cell and their CD28 counterligand are taken as evidence of antigen presentation and T cell activation. This interaction is demonstrable both in tissue sections and in functional studies where T cells and macrophages are cultured together (co-culture; Altaf 2007).

The first evidence of antigen presentation at the implant–bone interface came from the use of immunohistochemistry and MAB markers of the RFD series, one of which recognizes APCs (RFD1; Poulter *et al.* 1986). Thus, a proportion of macrophages and MNGCs were marked with RFD1 in samples of cases with aseptic loosening, and those having the highest percentages of RFD1-positive cells (70–90%) were those in which metal debris was a feature (Al-Saffar *et al.* 1997).

With the subsequent recognition of particular co-stimulatory molecules and the development of MABs against these, it has been possible to show that CD80 and CD86 are present on macrophages and MNGCs; also that CD28 is expressed by related T lymphocytes (Bainbridge *et al.* 2001; Farber *et al.* 2001; Altaf *et al.* 2003). The expression of CD86 predominates over that of CD80. It is interesting that the distribution in the implant interface of T lymphocytes and APC (RFD1-positive or CD80/86-expressing cells) is similar, namely, in the lower part of the synovium-like layer and in the perivascular region of the deeper tissue, suggesting close interrelationship between these cells.

Other co-stimulatory molecules found in the tissue at the bone–implant interface are CD40 present in APCs with its counterligand, CD40L, on lymphocytes (Bhatt *et al.* 2004), and the ICAM-1 and LFA-1 pairing that have also been shown on interface macrophages and T cells, respectively (Al-Saffar *et al.* 1994). That these co-stimulatory molecules are expressed by cells when they phagocytose particles has been shown in several different functional studies using cell culture methods (Bainbridge *et al.* 2001; Altaf *et al.* 2003; Altaf & Revell 2004; Altaf 2007).

Further evidence for active antigen presentation in the interface membrane of aseptic loosening comes from the investigation of transcription factor expression by macrophages and MNGCs in relation to wear debris (Altaf 2007). The response of all cells to external stimuli is by these intracellular signalling molecules that influence nuclear DNA and therefore the response of the cell, which may be the production of a protein. The various members of the NFκB family of molecules (namely, RelA, RelB, c-Rel, p50 and p52) were investigated quantitatively in cell culture studies using a macrophage cell line (U937) and peripheral blood monocyte/macrophages by RT-PCR and FACs analysis by Altaf (2007). She also examined the expression of these molecules by macrophages and MNGCs in tissue sections of interface tissues from cases of aseptic loosening. The molecule of particular relevance to the present discussion is RelB, which is expressed by APCs, and not other macrophages, during their activation in the process of phagocytosis and antigen presentation. The expression of RelB was clearly demonstrated by Altaf (2007) in macrophages of the interface inflammatory tissue as well as by cells engulfing wear debris *in vitro*.

9.4. Lymphocyte migration into the interface tissue

Most of the cells (lymphocytes and macrophages/MNGCs) in the interface tissues are there because they have migrated from local blood vessels, though it is known that some lymphocytes have proliferated *in situ*, as evidenced from Ki67 labelling (Revell & Jellie 1998). Even in the study of tissue sections, the recognition of the molecules expressed by endothelial cells lining the blood vessels can provide important information about the physiological processes taking place. The adhesion molecules involved in the process of cell migration are well described and may be readily detected by immunohistochemistry using MABs. Briefly, there is slowing of the flow of blood and cells fall out of the normally axial stream in the process called margination, which is followed by their attachment to the endothelial cells. Adhesion and transmigration follow. Attachment is under the influence of selectins, while adhesion and transmigration between the endothelial cells into the tissues involves integrins and CAMs. In the tissues at the implant interface in aseptic loosening, the endothelial cells of the vessels express P-selectin, E-selectin (ECAM-1), ICAM-1, VCAM-1 and CD44 (Al-Saffar *et al.* 1994; Clarke & Revell 2001; Altaf & Revell 2004; McFarlane & Revell 2004; Hercus 2005). The recognition of E-selectin expression is of particular importance in the context of lymphocyte function, for it is this molecule that has been shown to be present when T lymphocytes migrate from blood vessels at sites of cutaneous contact sensitization (Norris *et al.* 1992). In the context of aseptic loosening, it is posited that the demonstration of E-selectin provides further evidence that a sensitization process, most likely to metal, is involved in those cases where this molecule is present.

10. SYSTEMIC EFFECTS FROM THE DISSEMINATION OF WEAR PARTICLES AND THE PRODUCTS OF CORROSION TO DISTANT ORGANS

The detection of particles or metal ions in organs distant from the replaced joint has been described by various authors (Bauer *et al.* 1993; Case *et al.* 1994; Urban *et al.* 2000; Revell *et al.* 2004) and mentioned in §5 of this review. Metal ions have been shown by elemental analysis in the blood, synovial fluid and urine of individuals having replacement joints and the levels are higher where metal articulates against metal (Cracchiolo & Revell 1982; Dorr *et al.* 1990; Hart *et al.* 2006; Sargeant *et al.* 2006; Daniel *et al.* 2007). These high levels of metal are presumably bound to proteins and are probably derived by corrosion of the implant itself, including such items as fixing screws, or of wear particles. Metal against metal joints produce nanoparticles according to isolation studies (Doorn *et al.* 1998) and joint simulation experiments (Brown *et al.* 2007). Nanoparticles have a greater relative surface area than microparticles and are therefore more chemically reactive. This alone may be the reason for high metal ion levels in those with metal–metal replacement joints. In general, skin test positive contact sensitization is considered to be the result of metal ions acting as haptens, which is to say that although they are themselves too small to illicit an immune response, the combination of ions with larger molecules, namely proteins, enables immune processing to take place and for sensitization to result. Simple painting of the skin with soluble metal salts results in contact sensitization in experimental animals.

Reduced levels of T lymphocytes have been noted in the peripheral blood of those with metal implants in the absence of frank sensitization as judged by skin testing (Granchi *et al.* 1995; Hart *et al.* 2006). Concern expressed over the possibility of metal sensitization in patients receiving second-generation metal against metal joint replacements has already been mentioned. Park *et al.* (2005) described how 9 out of 165 patients having primary cementless metal–metal total hip replacements had an osteolytic lesion and these individuals had a higher incidence of cobalt sensitivity on patch testing than controls. In two of the cases, perivascular lymphocytes and macrophages were a feature of the histological picture in retrieved samples of periprosthetic tissue collected during revision arthroplasty. There are similar recent observations by Davies *et al.* (2005) and Willert *et al.* (2005). A wealth of material recorded by the author's own group over the last 15 years shows strong evidence for a T cell-mediated immunological reaction in aseptic loosening, as reviewed above. Samples examined were from cases with aseptic loosening in which the articulating surfaces were metal and PE, at the hip or knee. PE wear debris predominates under these circumstances and the presence of osteolysis has been directly correlated with the number of PE particles isolated from the tissue (Kobayashi *et al.* 1997). That various proteins including type I collagen, aggrecan, proteoglycans and immunoglobulins are bound to PE wear particles in aseptic loosening has been demonstrated (Wooley *et al.* 1999); that this has any relevance in terms of the generation of an immune reaction has not. Any altered response as a result of an immune reaction triggered by PE would likely be mounted against these proteins and fall into autoimmunity in the broadest sense of this term. This seems unlikely at present in the light of the available knowledge, albeit not extensive, about the comorbidity of joint replacement and autoimmune diseases.

In the context of metal–metal implants, there arises the question as to whether nanoparticles are more immunogenic than microparticles. The toxicity of nanoparticles in general has been reviewed recently (Revell 2006*a*) and it seems that some materials are toxic causing malignancy and others are relatively harmless. Little or no information is yet available about nanoparticles of the metal alloys used in joint replacement, and the lessons from other materials show the dangers of extrapolation in this area. For example, carbon nanoparticles, as carbon black or diesel fume, are markedly toxic, while nanoparticulate carbon in diamond form is less toxic than microparticles of diamond (Altaf *et al.* 2006; Revell 2006*a*; Altaf 2007). No direct comparison has been made in the same cell culture experiment but it is very likely that there are clear differences between different forms of nanoparticulate carbon. While this might seem a diversion from the main theme of the current article, it does point up the need for future careful comparative studies of the effects of different metals in nano- and microparticulate forms before conclusions are drawn as to the toxicity of metal nanoparticles.

There is a lack of information about whether sensitization to a metal can exist separately from contact sensitization as demonstrated by skin testing. The assumption in the orthopaedic community has been that the lack of a positive skin test against a known sensitization agent means that sensitization is not a factor in the causation of implant loosening. This has always seemed an oversimplification in the opinion of the author, who has held that sensitization may originate in major lymphoid organs such as the spleen and be systemically (centrally) expressed rather than in the skin. If this were true, the response to the sensitizing agent might occur where the material was present, deep in the bone around the implant and not necessarily on the skin. Again, there is currently insufficient information about this putative systemic form of sensitization.

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